Supplemental Material

Method:

Quantitative RT-PCR Confirmation of Gene Changes. Quantification of the eight selected transcripts was performed by relative quantitative real-time RT-PCR with TaqMan real-time RT-PCR assays and 7900HT Fast Real-time PCR system (Applied Biosystems, Foster City, CA). cDNAs were generated from 1 μg of total RNA using SuperScript III First-Strand Synthesis SuperMix kit (InVitrogen, Carlsbad, CA). The resulting cDNA was subjected to a 14 cycle PCR amplification using manufacturer's TaqMan PreAmp Master Mix Kit protocol. The ready-made primer and probe sets were ordered from Applied Biosystems. (Catalog #: *Tff2*: Mm00447491_m1; *Clca3*: Mm00489959_m1; *Rgs9*: Mm00599991_m1; *Ear11*: Mm00519056_s1; *Cfb*: Mm00433923_g1; *Tlr2*: Mm00442346_m1; *Musin5b*: Mm00466395_g1; *Gapdh*: Mm99999915_g1). Three replicates were run for each gene for each sample in a 384-well plate. Glyceraldehyde-3-phosphate dehydrogenase was used as the endogenous reference gene as it does not exhibit significant expression changes between four groups of samples. The relative quantitation method (ΔΔCt) was used, with the ratio of the mRNA level for the gene of interest normalized to the level of internal control and the average of the PBS control samples as the calibrator value. The significance of the TaqMan data was calculated and significance was considered when $p < 0.05$.

Result

Validation of Differentially Expressed Genes by qRT-PCR. The differences in gene expression found by microarray analyses were validated by using quantitative real time RT-PCR (qRT-PCR). We chose genes (*Tff2, Clca3, Rgs9, Ear11, Cfb, Tlr2, Mus5b and Cxcl2*) with

greater than 3-fold regulated expression according to our microarray results. Although some variation concerning the degree of regulation was observed, the data obtained with microarrays were substantially confirmed for all genes by qRT-PCR (Supplemental Material Figure 1).

Figure Legend

Supplemental Material Figure 1. Microarray validation of gene targets by rtPCR. Differentially expressed gene levels (from both microarray chip intensity and qRT-PCR) by treatment of PM, OVA or OVA+PM compared to PBS control levels (in fold change or log fold change) of selected genes. ***Muc5b***, mucin 5, subtype B; ***Tff2***, trefoil factor 2; ***Rgs9***, regulator of G-protein signaling 9; ***Cfb***, complement factor B; **TLR2**, toll-like receptor 2; ***Cxcl2***, chemokine (C-X-C motif) ligand 2; ***Ear11***, eosinophil-associated, ribonuclease A family, member 11; ***Clca3***, chloride channel calcium activated 3.

Supplemental Material Table 1: Gene filtering criteria and result by SAM

Gene list	Pairwise comparison	Delta	False positives	FDR (%)	Fold change	Significant probe sets	Unique gene	Unique Up	Unique Down
1	OVA vs. Control	1.53	2.68	6.86	2	39	37	21	16
2	PM vs. Control	1.5	2.41	0.45	3	539	437	374	63
3	OVA//PM vs. Control	1.8	0.48	0.07	3	719	591	492	99
4	OVA/PM vs. PM	0.6	6.74	4.78	2	149	127	72	55

Supplemental Material Table 2. PM-induced Top 25 Most Differentially Regulated Genes*.

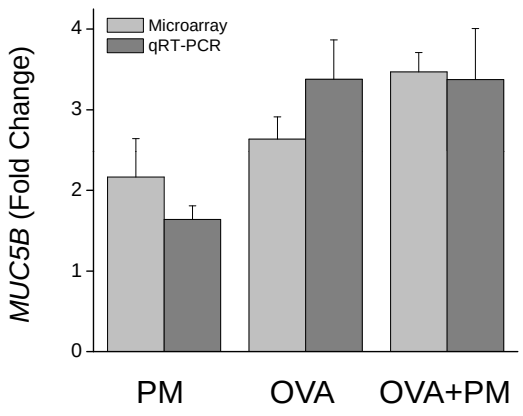
Probe set ID	Gene Symbol	Gene Title	Fold Change
1427381_at	*Irg1*	immunoresponsive gene 1	339.0264
1438148_at	*Gm1960*	gene model 1960, (NCBI)	324.9832
1450826_a_at	*Saa3*	serum amyloid A 3	155.6622
1418652_at	*Cxcl9*	chemokine (C-X-C motif) ligand 9	120.6404
1449984_at	*Cxcl2*	chemokine (C-X-C motif) ligand 2	108.959
1418930_at	*Cxcl10*	chemokine (C-X-C motif) ligand 10	105.5197
1419561_at	*Ccl3*	chemokine (C-C motif) ligand 3	95.77347
1449153_at	*Mmp12*	matrix metallopeptidase 12	92.33373
1449452_a_at	*Gp2*	glycoprotein 2 (zymogen granule membrane)	69.64431
1434046_at	*AA467197*	expressed sequence AA467197	68.5962
1418287_a_at	*Dmbt1*	deleted in malignant brain tumors 1	65.46579
1416306_at	*Clca3*	chloride channel calcium activated 3	63.82462
1421008_at	*Rsad2*	radical S-adenosyl methionine domain containing 2	62.49211
1425890_at	*Ly6i*	lymphocyte antigen 6 complex, locus I	58.96273
1423017_a_at	*Il1rn*	interleukin 1 receptor antagonist	51.43086
1451905_a_at	*Mx1*	myxovirus (influenza virus) resistance 1	47.08504
1420330_at	*Clec4e*	C-type lectin domain family 4, member e	42.83071
1460227_at	*Timp1*	tissue inhibitor of metalloproteinase 1	41.37069
1418165_at	*Itlna*	intelectin a	39.69577
1423555_a_at	*Ifi44*	interferon-induced protein 44	37.84375
1419482_at	*C3ar1*	complement component 3a receptor 1	31.73106
1424339_at	*Oasl1*	2'-5' oligoadenylate synthetase-like 1	30.27398
1419282_at	*Ccl12*	chemokine (C-C motif) ligand 12	27.56718
1421596_s_at	*H28*	histocompatibility 28	26.59342
1419684_at	*Ccl8*	chemokine (C-C motif) ligand 8	25.77237

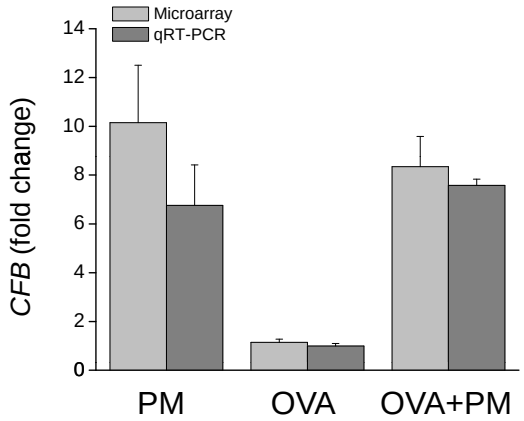
* All genes are significant (q-value < 0.1%); and the full gene list is in our website (http://phenos.bsd.uchicago.edu/publication/PMAsthma/).

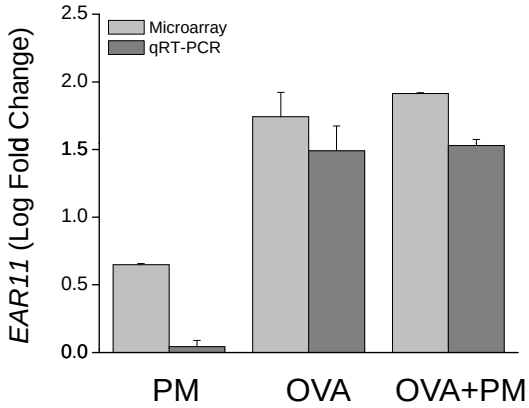
Supplemental Material Table 3: OVA/PM-induced dysregulated genes in ion transport (GO:0006811)*

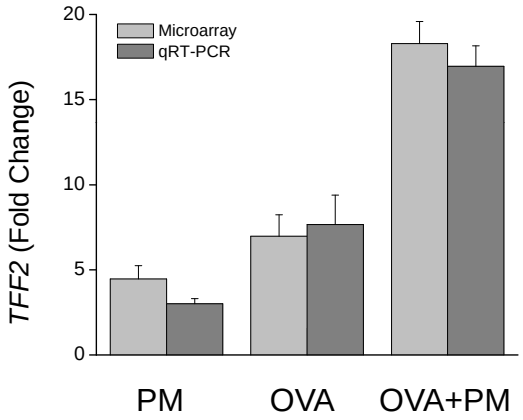
Accession	UniGene ID	Symbol	Gene ID	Fold
BB769890	19298	*Atp6v0d2*	242341	4
AV348121		*Slco4a1*	108115	5.3
BG176150	333349	*Prkwnk1*	232341	-4.1
NM_020574	282386	*Kcne3*	57442	3
NM_033648	250392	*Fxyd4*	108017	15.3
BB009037	13787	*Cp*	12870	4.5
AV371434	25237	*Slc5a1*	20537	18.2
NM_054098	31403	*Tnfaip9*	117167	3.2
BC027114	44101	*Atp1a3*	232975	3.2
AF297098	85429	*Steap*	70358	3.2
NM_013612	2913	*Slc11a1*	18173	6.2
BC021548	28804	*0610039P13Rik*	74096	4.5
NM_031176	290527	*Tnxb*	12723	-3.5
NM_007504	35134	*Atp2a1*	11937	-20.1
BG865910	9911	*Kcnn4*	16534	3.4
U31908	388924	*Kcnab2*	16498	3.1
AK003626	250980	*Slc38a4*	69354	-3.1
NM_008165	4920	*Gria1*	14799	-5.2
NM_008522	282359	*Ltf*	17002	6.1

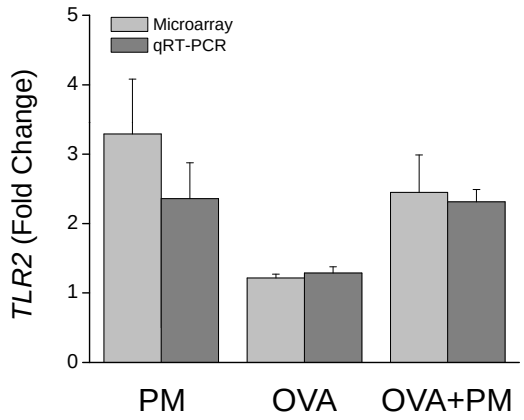
* The significance of dysregulation was determined by SAM analysis; the GO category was identified by OntoExpress software (see Table 2).

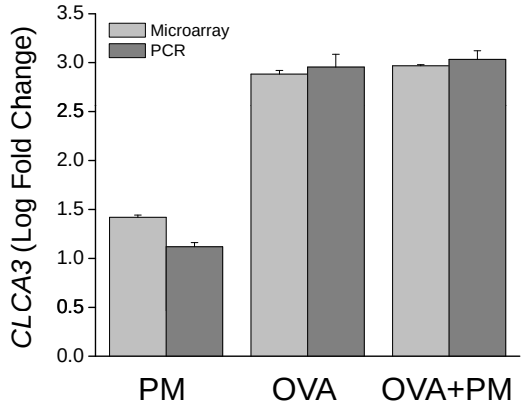


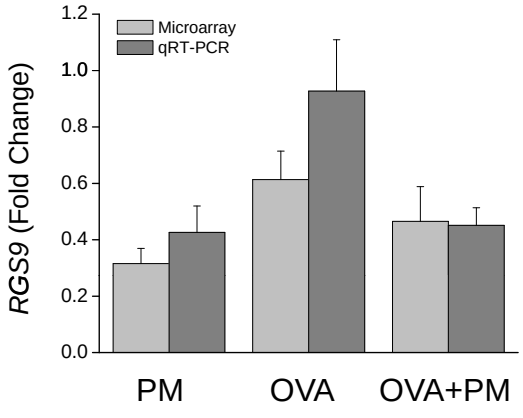


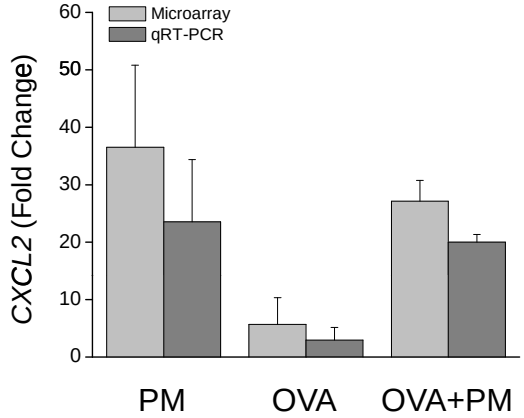












Supplemental Material Figure 1